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**Cornell Entomologists
Demonstrate Better Insect
Control with Novel Technique of
"Gene Pyramiding"**

by Peter Seem

GENEVA, NY: Entomologists at Cornell University have provided the first experimental evidence that breeding plants to produce two different proteins by a process called "gene pyramiding" delays the development of resistance in targeted insect pests. The research has important implications for the long-term protection of agricultural crops produced through biotechnology, particularly Bt corn and Bt cotton. The team performed the research using diamondback moths, one of the world's major insect pests, and Bt broccoli.

The paper, "Transgenic plants expressing two *Bacillus thuringiensis* (Bt) toxins delay insect resistance evolution," will be published in the journal *Nature Biotechnology* on December 1.

Bt, or *Bacillus thuringiensis*, is a type of bacterium that produces proteins toxic to many major agricultural insect pests. Bt was promoted as an environmentally benign insecticide by Rachel Carson in her 1962 book, *Silent Spring*. Even though it is benign, Bt accounts for less than two percent of the world's insecticides because of its cost and relatively low effectiveness. When plant breeders developed the technology to genetically engineer the gene for Bt into a specific crop, the crop itself became a very effective method of control. Bt plants were first commercialized in 1996, and Bt corn and Bt cotton became widely used alternatives to conventionally bred corn and cotton. In 2002, Bt crops were grown on 36 million acres worldwide.

"Breeding plants to express Bt proteins provides positive economic benefits to growers, and health benefits for the environment and farm workers," said Tony Shelton, Cornell University professor of entomology at the New York State Agricultural Experiment Station, in Geneva, NY, and one of the paper's authors. "We're moving into the second generation of the technology now. As techniques have become more sophisticated, technology allows us to pyramid two Bt genes in a plant."

The paper is the result of 10 years of research by Shelton and his collaborators to develop transgenic plants as an alternative to conventional insecticide sprays. Using dual-toxin broccoli plants developed by Elizabeth Earle and Jun Cao, in the plant breeding department at Cornell, Shelton's lab examined how resistance to the two toxins developed in a population of diamondback moth after 24 generations. Resistance was compared under several different management strategies.

"Plants containing two Bt toxin genes substantially delayed the development of resistance compared to two single-toxin plants used sequentially or in a mosaic," said Shelton. "Regulatory agencies and companies now



Suggested caption: The principle scientists involved with the Bt pyramid gene project are (inset photo - left to right) Jun Cao and Lisa Earle (Plant Breeding, Ithaca), Tony Shelton and Jian-Zhou Zhao (Entomology, Geneva). The background photo, taken in the greenhouse in Geneva, NY, shows some of the large cages used in the tests with Bt broccoli.

Credit: J. Ogradnick/NYSAES/Cornell

should work together to promote the development of these pyramid plants and, in the long term, phase out single gene plants."

Mathematical models of insect resistance suggest that plants with genes for two different Bt toxins would delay resistance longer than planting a mixture of two single-toxin plants in the field (called a mosaic), or using two single-toxin plants sequentially in crop rotation. Such models have already prompted one company to develop a variety of cotton that expresses two Bt proteins. Shelton's lab provides the first experimental confirmation of the value of dual-toxin plants.

Since the commercialization of Bt plants for insect control, there have been no instances of insect populations developing resistance in the field, but there is a constant danger that the pest species will develop resistance to the toxin, as has happened with many conventional insecticides. To help prevent insects from developing resistance to transgenic Bt crops, the EPA has mandated that a portion of acreage next to a Bt crop be devoted to what is called a "refuge." A refuge is an area in which the non-transgenic version of the crop is grown. This area allows some susceptible insects to survive so that the gene that encodes for resistance does not become abundant in the insect population.

In addition to providing better resistance management, plants with pyramided genes for Bt proteins require less space set aside as refuge, which helps growers (for whom a refuge can represent a significant portion of the crop damaged) get a greater return for their acreage. Preventing insect resistance also extends the useful life of Bt crops, which helps manufacturers and growers.

"We have based resistance management programs in the United States on some pretty solid theory, but Shelton and his team have given us very useful data," said Fred Gould, professor of entomology at North Carolina State University, and a leading expert on Bt crops. "This research should make the EPA and companies more open to developing pyramided or dual-toxin plants."

"This work has important implications in the U.S., but also in Australia, India and China, where millions of acres of the cotton crop contains Bt," adds Jian-Zhou Zhao, the paper's first author.

"Using more Bt crops and less insecticide are environmentally and people friendly strategies in pest control," said Shelton. "The next step in our research program is to extend the crop's useful life by having plants express the Bt proteins only when the crop is most susceptible to insect damage."

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